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TF/VIIa-dependent Factor X Activation

TF/VIIa/phospholipids/ Ca^{++} + DMSO (diluted DMSO or Buffer)

or Compound

15 min at 37 C



Add FX



Add EDTA



Add FXa Substrate



Add 50% Acetic Acid



Read OD at 405nm

FIG. 1



Table 1

Compounds	% Inhibition of FXa Activity at		% Inhibition of FVIIa Activity at		% Inhibition of Thrombin Activity at		% Inhibition of Trypsin Activity at	
	714 μ M	71 μ M	833 μ M	83 μ M	625 μ M	63 μ M	625 μ M	63 μ M
Compound <u>2</u>	3	3	27	5	57	-16	-8	-16
Compound <u>1</u>	7	1	42	1	41	-9	-2	10

Compounds were in DMSO and were diluted with 10 mM HEPES-NaOH. pH 7.5 if necessary

FIG. 2A

Table 2

Compounds	% Inhibition of FX Activation Catalyzed by			
	TF/VIIa	RVV	FIXa	FVIIa
Compound 2 96 μ M 32 μ M	nd ²	6.1	23.2	nd
	94.5	1.8	6.8	nd
Compound 1 100 μ M 96 μ M 32 μ M	nd	nd	nd	6.0
	nd	5.0	21.7	nd
	85.0	4.9	0.0	nd

nd² . not determined.

The compounds were in DMSO and were diluted with 10 mM Hepes-NaOH.pH 7.5 if necessary

FIG. 2B



Table 3

Compound Concentrations (μ M)	% Inhibition by Compound <u>2</u>	% Inhibition by Compound <u>1</u>
0	0	0
2	6	1
4	10	7
8	45	14
16	62	42
32	84	83
48	92	91
96	96	94

Compounds 1 and 2 were dissolved in 0.1 M NaOH, then diluted to 16.5 mM NaOH with water. The compounds were preincubated with TF/VIIa for 15 minutes at 37 C prior to addition of FX. Following FX addition, the reaction was incubated for 15 minutes at 37 C.

FIG 2C



Table 4

Compound Concentrations (μ M)	% Inhibition by Compound <u>2</u>	% Inhibition by Compound <u>1</u>
0	0	0
2	24.7	
4	39.9	-0.3
8	67.2	20.8
16	79.4	26.9
32	87.5	61.1
48		84.5
96		90.3

Compounds 1 and 2 were in DMSO and diluted with 10 mM Hepes-NaOH, pH 7.5

FIG. 2D



Compounds	Factor X Activation IC ₅₀ in μ M	Factor IX Activation IC ₅₀ in μ M	FXa Activity % Inhibitors @ 83 μ M	FVIIa Activity % Inhibitors @ 83 μ M	Thrombin Activity % Inhibitors @ 63 μ M	Trypsin Activity % Inhibitors @ 71 μ M
Compound <u>1</u>	10	26	4	1	0	4
Compound <u>2</u>	6.5	55	3	5	0	0
Compound <u>3</u>	70	n.d.	n.d.	n.d.	n.d.	n.d.
Compound <u>4</u>	>200	n.d.	n.d.	n.d.	n.d.	n.d.
Compound <u>5</u>	100	n.d.	n.d.	n.d.	n.d.	n.d.
Fosamax	80	n.d.	n.d.	n.d.	0	n.d.

n.d. – not determined

FIG. 3



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Compounds	PT Clotting Time (Seconds)	APTT Clotting Time (Seconds)
Control (DMSO)	37.0	29.4
Compound 1 (167 μ M)	75.3	35.4
Compound 2 (167 μ M)	76.8	33.0

FIG. 4



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Compound	Km for FX (nM)
Control (DMSO)	19
10 μ M - compound <u>1</u> (in DMSO)	37
20 μ M - compound <u>2</u> (in DMSO)	76

FIG. 5

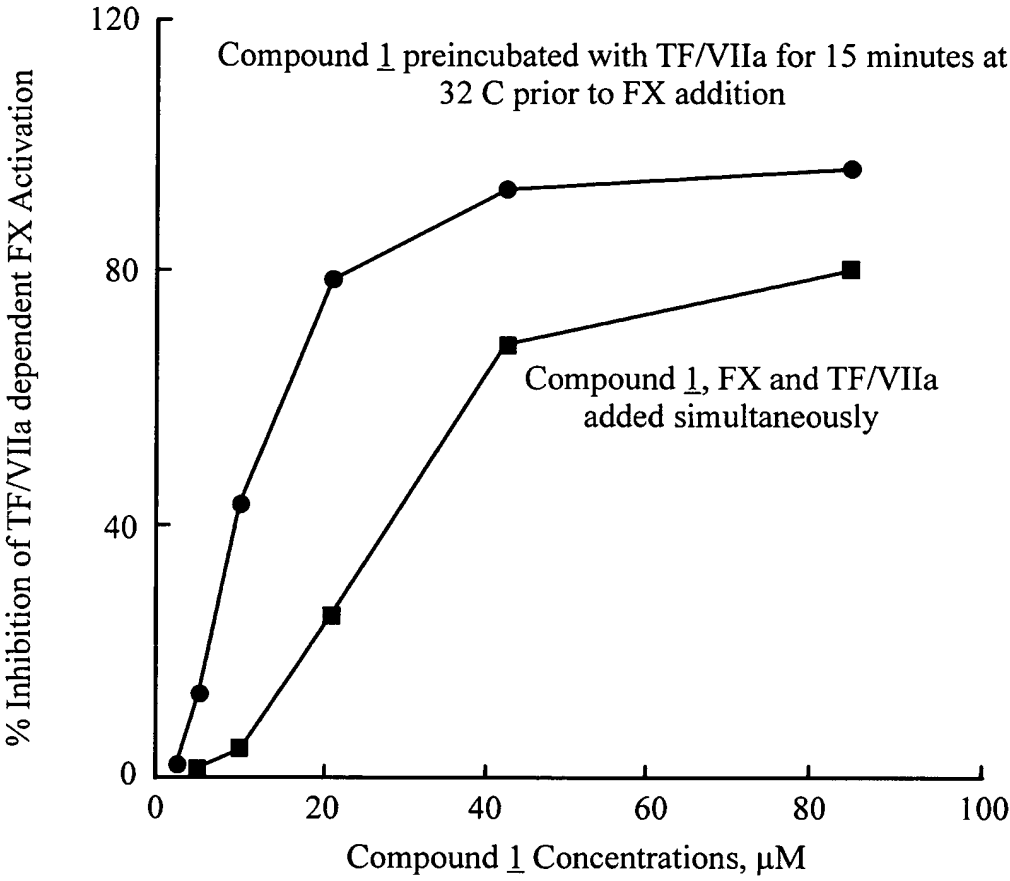


FIG. 6

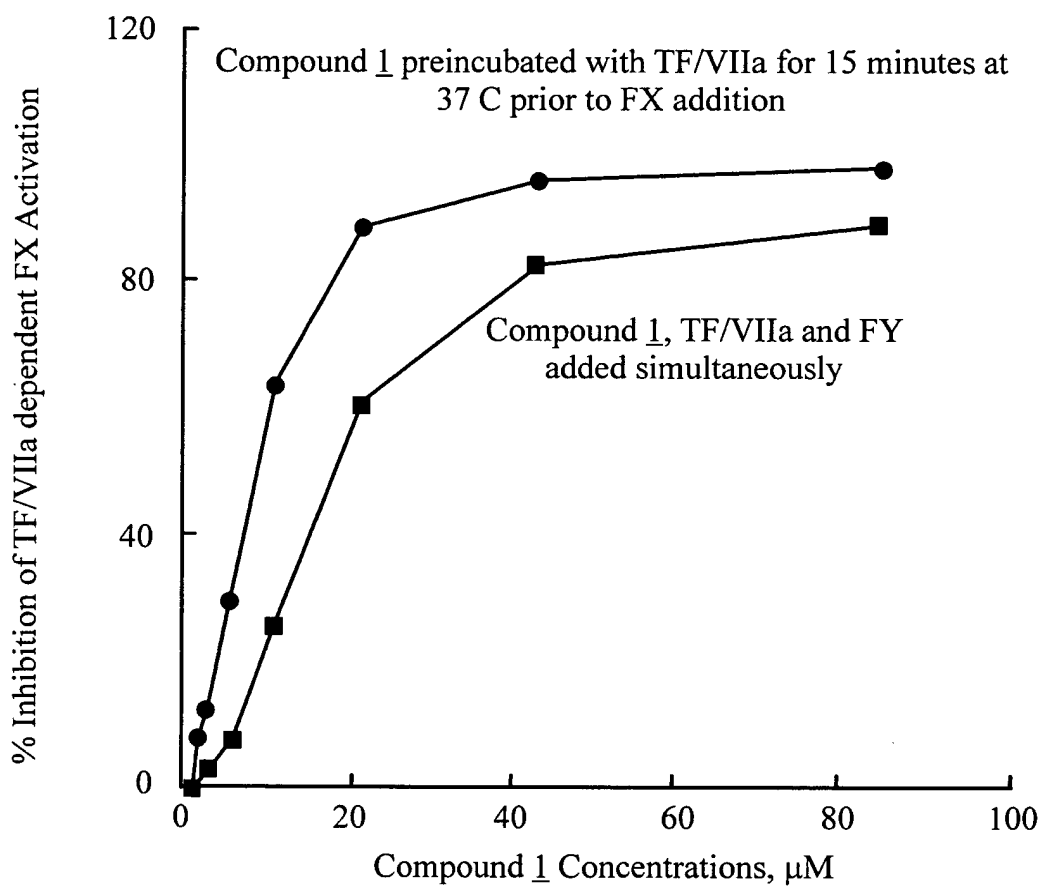


FIG. 7